REFERENCES

Chun, T.W., Moir, S., and Fauci, A.S. (2015). Nat. Immunol. *16*, 584–589.

Ciechonska, M., and Duncan, R. (2014). J. Virol. *88*, 6528–6531.

Cohen, F.S., and Melikyan, G.B. (2004). J. Membr. Biol. *199*, 1–14.

Inuzuka, M., Hayakawa, M., and Ingi, T. (2005). J. Biol. Chem. *280*, 35776–35783.

Matheson, N.J., Sumner, J., Wals, K., Rapiteanu, R., Weekes, M.P., Vigan, R., Weinelt, J., Schindler, M., Antrobus, R., Costa, A.S.H., et al. (2015). Cell Host Microbe *18*, 409–423.

Rosa, A., Chande, A., Ziglio, S., De Sanctis, V., Bertorelli, R., Goh, S.L., McCauley, S.M., Nowosielska, A., Antonarakis, S.E., Luban, J., et al. (2015). Nature *526*, 212–217.

Tokarev, A., and Guatelli, J. (2011). Cell. Logist. *1*, 90–102.

Usami, Y., Wu, Y., and Göttlinger, H.G. (2015). Nature *526*, 218–223.

Varoqui, H., Zhu, H., Yao, D., Ming, H., and Erickson, J.D. (2000). J. Biol. Chem. *275*, 4049–4054.

Waheed, A.A., and Freed, E.O. (2010). Viruses *2*, 1146–1180.

Wang, R., Dillon, C.P., Shi, L.Z., Milasta, S., Carter, R., Finkelstein, D., McCormick, L.L., Fitzgerald, P., Chi, H., Munger, J., and Green, D.R. (2011). Immunity *35*, 871–882.

Salmonella Manipulates Autophagy to "Serve and Protect"

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Many intracellular pathogens, including *Salmonella typhimurium*, trigger autophagy in host cells, which is widely thought to restrict intracellular growth and survival. In this issue of *Cell Host & Microbe*, Kreibich et al. (2015) demonstrate a role for the autophagic machinery in the repair of damaged *Salmonella*-containing vacuoles (SCVs).

Macroautophagy (herein referred to as autophagy) is a highly conserved intracellular process that delivers unwanted or damaged cytoplasmic components to lysosomes for degradation. This process involves the complex interplay of more than 36 autophagy-related (ATG) proteins that work in a coordinated manner to enclose cytoplasmic material in a double-membrane-bound vacuole (the autophagosome), which then fuses with lysosomes to form degradative autolysosomes. In this manner, autophagy functions as a quality control mechanism, eliminating protein aggregates and damaged organelles to maintain cellular homeostasis (Mostowy, 2013). However, many studies over the last 10 years indicate that cells can also utilize autophagy for the elimination of invading pathogens (Cemma and Brumell, 2012).

Indeed, elegant studies of *Listeria*, *Shigella*, *Mycobacteria*, and *Salmonella* pathogenesis underscore the important role played by antibacterial autophagy (xenophagy) in the restriction of bacterial replication (reviewed in Cemma and Brumell, 2012). Of these, *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) is perhaps the best understood. *Salmonella* express two Type Three secretion systems (T3SSs) encoded by *Salmonella* pathogenicity islands (SPI)-1 and -2. These needle-like complexes penetrate host membranes and are used to deliver distinct arrays of bacterial effectors into host cells. While the SPI-1 T3SS is required for active invasion, expression of the SPI-2 T3SS promotes intracellular survival within a modified phagosomal compartment called the *Salmonella*-containing vacuole (SCV). After the early stages of infection, the SPI-1 system is generally downregulated, and the phagosomal environment (low pH, low Mg^{2+} and Fe^{3+} content, low nutrient availability) triggers expression of the SPI-2 TTSS (for review, see Figueira and Holden, 2012). Despite their differential regulation, expression of SPI-1 and SPI-2 T3SS effector proteins can overlap, and cooperate in forming the SCV (Agbor and McCormick, 2011).

As a mechanism of antibacterial defense, autophagy appears to target *Salmonella* in two ways. While most bacteria are contained within SCVs, a small proportion of *Salmonella* escapes the vacuole and can hyperreplicate in the cytosol. These cytosolic *Salmonella* do not go unnoticed, and are tagged by ubiquitin soon after their release from the SCV (Figure 1, pathway #1). Ubiquitylation leads to the recruitment of a variety of autophagy adapters including SQSTM1/p62, NDP52 (nuclear dot protein 52 kDa), and optineurin (OPTN) (Mostowy, 2013), which bind to ubiquitylated bacteria via an ubiquitin (Ub)-binding domain and link them to nascent autophagosomes through their interaction with an autophagosomal membrane-anchored member of the ATG8 family (LC3). Once sequestered within a completed autophagosome, bacteria are killed within the

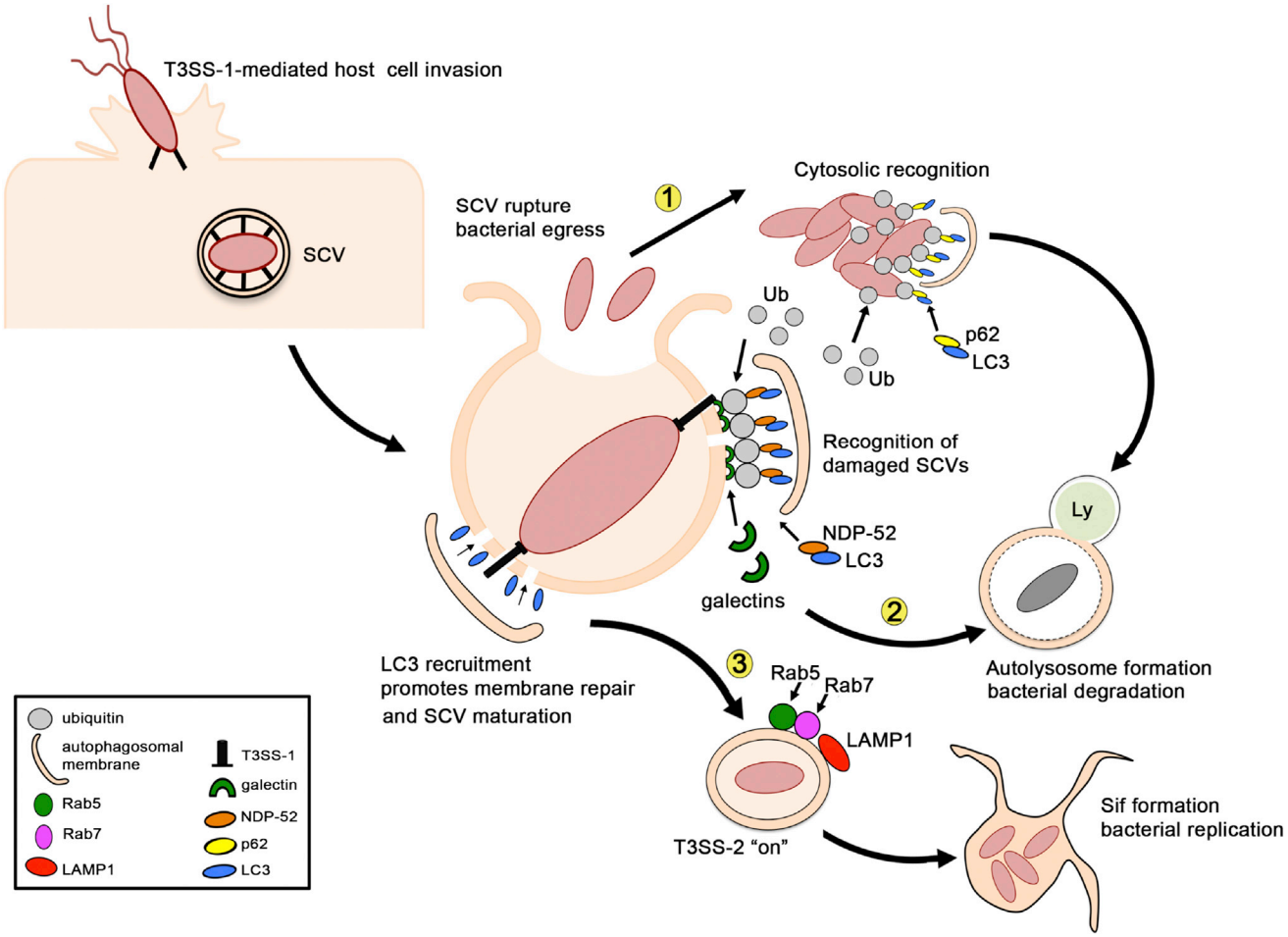

Figure 1. *Salmonella* "Seals" Its Fate

After internalization by host cells, *Salmonella* resides within a modified phagosomal compartment called the *Salmonella*-containing vacuole (SCV). While expression of the Type III secretion system T3SS-1 is required for invasion, this needle-like apparatus can cause damage to the SCV, resulting in multiple consequences for the pathogen. (1) If T3SS-mediated damage is sufficient, the SCV can rupture, allowing bacterial egress into the cytosol. Cytosolic *Salmonella* are rapidly tagged with ubiquitin and marked for autophagy by adaptor molecules including p62 (pictured), NDP52, and OPTN. Autophagy receptors interact with Atg8 family members, such as LC3, to promote autophagosome biogenesis. (2) *Salmonella* residing in damaged SCVs can also be targeted by autophagy. In this case, modified carbohydrate structures on the SCV recruit galectins, NDP-52, and ubiquitin to the membrane, which tag these compartments for autophagic elimination. (3) In contrast to antibacterial autophagy, *Salmonella* can utilize autophagy machinery to help seal leaky SCVs. This allows for the sequential acquisition of Rab5 followed by Rab7 to ensure compartment maturation and T3SS-2 expression, which together facilitates intracellular survival.

hydrolytic environment produced by the fusion of the bacteria-containing autophagosome with a lysosome (Mostowy, 2013).

The second way *Salmonella* are targeted by autophagy involves the recognition of damage to the SCV resulting from the pore-forming properties of the SPI-1 T3SS. In this case, *Salmonella* do not need to be free in the cytosol for autophagic capture and degradation to occur (Birmingham et al., 2006). Perforation of the SCV membrane by T3SS-1 leads to recruitment of cytosolic galectins including galectin-8, which serve as monitors of endolysosomal integrity (Thurston et al., 2012). SCVs decorated with galectin-8 recruit ubiquitin ligases and NDP-52 to the membrane, thereby marking these compartments for autophagic elimination (Figure 1, pathway #2).

The ability of host cells to recognize damaged SCVs poses a logistical problem for the bacterium; while T3SS-1 is necessary for invasion of epithelial cells and the early stages of SCV biogenesis, the damage it inflicts on host cell membranes can alert the host to its presence and trigger an antibacterial autophagic response.

In this issue of *Cell Host & Microbe*, Kreibich et al. (2015) provide evidence for an unexpected role for autophagy: rather than functioning in a strictly antibacterial capacity, the authors demonstrate that the autophagic machinery can actually *repair* damage to SCV membranes caused by T3SS-1, thereby allowing compartment maturation and subsequent expression of T3SS-2, which together promote intracellular survival.

Using an imaging-based RNAi screening platform, the authors set out to identify host factors involved in the induction of T3SS-2, which occurs only when *Salmonella* are in a mature, intact SCV. As a reporter for SCV maturation, they used an *S. typhimurium* strain expressing GFP under control of a T3SS-2 promoter (*ssaG*) (Schlumberger and Hardt, 2006). Since T3SS-2 is not

expressed by bacteria that have escaped into the cytosol or reside in damaged vacuoles, this strategy allowed them to focus specifically on factors that promote SCV biogenesis rather than bacterial proliferation per se.

As expected, this screen identified multiple well-established regulators of SCV maturation including the endosome-associated GTPases Rab5 and Rab7, and the vacuolar ATPase. Surprisingly, however, it also identified a large number of autophagy-related genes, including components that act as regulators (mTOR), recruitment factors (galectins, optineurin), initiation factors (Beclin, ULK-1, PI3-kinase), and the ATG8 and ATG12 conjugation systems. Given the important role of autophagy in the elimination of intracellular bacteria, it was initially unclear how this system could also be involved in supporting SCV biogenesis. By focusing solely on ssaG expression, the authors observed that loss of Atg5 (a component of the ATG8 conjugation system) actually *suppressed* expression of T3SS-2 and the growth of bacteria *within* SCVs. These observations strongly suggested that SCV maturation, as determined by T3SS-2 induction, is actually *dependent* on the autophagic machinery.

Why would this be? As noted above, previous studies have shown that T3SS-1 can perforate the SCV membrane, presumably through insertion of the translocon pore. Such leaks in the SCV membrane would result in dissipation of the proton gradient required for compartment acidification (and therefore for T3SS-2 induction). Effective repair of the vacuolar membrane would allow reacidification of the lumen and restoration of an environment permissive for T3SS-2 expression. In agreement with this hypothesis, the authors found that maturation of SCVs containing T3SS-1-deficient bacteria did not require Atg5. Conversely, they showed that retention of a fluid-phase marker (FITC-dextran) by SCVs containing T3SS-1-competent bacteria was more efficient in cells expressing Atg5 than in Atg5-deficient cells. In an interesting twist to the narrative, Atg5 was also required to repair endosomal membrane damage caused by osmotic shock, suggesting a more general role for autophagy in maintaining endosome stability.

Based on these data, the authors propose a working model whereby T3SS-1 damage to the SCV results in recruitment of the Atg12 conjugation system, which is essential for the repair and resealing of compromised membranes. The repair process restores SCV acidification, maturation, and ultimately T3SS-2 expression (Figure 1, pathway #3). In the absence of Atg5 (an essential component of the Atg12 conjugation system), lack of SCV repair leads to stalled vacuolar maturation, bacterial egress from the vacuole and cytosolic hyperproliferation.

This work is intriguing, not only because it uncovers a function for autophagy in bacterial pathogenesis, but also because it raises a multitude of questions about *Salmonella*-host interactions. From the perspective of the pathogen, it is understandable that *Salmonella* would prefer to replicate undetected in an SCV rather than in the cytosol, where it is subject to autophagy as well as a plethora of other antimicrobial mechanisms. But what tips the balance between autophagic recognition of the damaged SCV leading to elimination versus recognition leading to repair? It is possible that the fate of the SCV depends on the level of damage sustained, with heavily compromised SCVs targeted for autophagic capture and degradation, and those with less damage undergoing repair.

An important unanswered question is how the autophagic machinery actually repairs the vacuolar membrane. Are double-membraned autophagosome precursors (phagophores) somehow involved, or is repair driven by a distinct mechanism? Is it possible that T3SS-1 effectors themselves promote the repair process? An interesting candidate effector molecule is SptP, which is implicated in early SCV biogenesis. SptP is a bifunctional protein that functions both as a GTPase activating protein (GAP) and as a tyrosine phosphatase (Humphreys et al., 2009). SptP dephosphorylates valosin-containing protein (VCP), a member of the AAA protein family that functions in a variety of physiological processes including endosomal sorting and homotypic membrane fusion (Humphreys et al., 2009). However, while VCP and its adaptors are required for efficient SCV maturation, their functions during this process have not been elucidated. Interestingly, it has been shown that VCP associates with ubiquitin (Ub) machinery and is essential for the maturation of Ub-containing autophagosomes (Tresse et al., 2010). Therefore, further investigations will provide exciting insights into the mechanistic nature of autophagy-mediated SCV sealing and how this potentially impacts the course of infection in in vivo models.

REFERENCES

Agbor, T.A., and McCormick, B.A. (2011). Cell. Microbiol. *13*, 1858–1869.

Birmingham, C.L., Smith, A.C., Bakowski, M.A., Yoshimori, T., and Brumell, J.H. (2006). J. Biol. Chem. *281*, 11374–11383.

Cemma, M., and Brumell, J.H. (2012). Curr. Biol. *22*, R540–R545.

Figueira, R., and Holden, D.W. (2012). Microbiology *158*, 1147–1161.

Humphreys, D., Hume, P.J., and Koronakis, V. (2009). Cell Host Microbe *5*, 225–233.

Kreibich, S., Emmenlauer, M., Fredlund, J., Rämö, P., Münz, C., Dehio, C., Enninga, J., and Hardt, W.-D. (2015). Cell Host Microbe *18*, this issue, 527–537.

Mostowy, S. (2013). Cell. Microbiol. *15*, 395–402.

Schlumberger, M.C., and Hardt, W.D. (2006). Curr. Opin. Microbiol. *9*, 46–54.

Thurston, T.L., Wandel, M.P., von Muhlinen, N., Foeglein, A., and Randow, F. (2012). Nature *482*, 414–418.

Tresse, E., Salomons, F.A., Vesa, J., Bott, L.C., Kimonis, V., Yao, T.P., Dantuma, N.P., and Taylor, J.P. (2010). Autophagy *6*, 217–227.